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Long Term Memory and Morphological Distribution of Involved Proteins in Goldfish Brain

The turnover rates of three goldfish brain proteins are specifically enhanced when the animals learn a new swimming behaviour (SHASHOUA 1976): The fish are trained to swim with a polystyrene float sutured to their ventral skin. After removal of the floats experimental animals are injected with ^{3}H-valine into the IV. brain ventricle, whereas control fish obtain injections of ^{14}C-valine. Four hours later the brains of all fish are dissected, combined, homogenized together and separated into subcellular fractions. The proteins of these fractions are further separated by electrophoresis on SDS-polyacrylamide gels. In the cytoplasmic fraction three specific proteins, α, β and γ, migrating at apparent molecular weights of 37,000, 32,000 and 26,000 daltons, show an enhanced incorporation of radioactivity after training. Animals which do not succeed to learn the task, exhibit no increased rate of synthesis of the described proteins.

β and γ are glycoproteins. They were isolated by a combination of affinity chromatography on concanavalin A and SDS-polyacrylamide gel electrophoresis, purified and used to immunize rabbits. The antisera obtained were tested for their ability to interfere with learning. Although they do not prevent the acquisition of the new swimming pattern, they inhibit long term memory of the acquired behaviour, when they are injected into the IV. brain ventricle during a critical time period after training (SHASHOUA and MOORE 1978).

BENOWITZ and SHASHOUA (1977) have used anti-β antiserum for immunohistochemical localization of the antigen. They reported immunoreactivity in cells of the ependymal zone, especially abundant in the optic tectum, the dorsal tegmentum and the vagal lobes, and suggested the name ependymin β for the antigen.

We have further investigated the morphological distribution of ependymin β and γ using several different antisera, staining procedures and fixatives. In addition to immunoreactivity distributed along the whole length of the ependyma, we observe positive staining in the meninges and the granular layers of the optic tectum, the diencephalon and the vagal lobes. In cryostate sections of unfixed brains quickly frozen in liquid nitrogen antigenic material also occurs extracellularly, especially in the perivascular space. No difference was observed in the distribution of ependymin β and γ.

In order to analyse the distribution of the protein in a quantitative manner, ependymin β was labeled with ^{125}I and used to develop a very sensitive radioimmunoassay (SCHMIDT and SHASHOUA 1981). The assay is specific for ependymin β, i.e. it does not cross-react with other proteins, including several glycoproteins isolated from goldfish brain. Full cross-reactivity was observed, however, with ependymin γ, the other glycoprotein exhibiting an enhanced rate of synthesis after training.

The radioimmunoassay was applied to demonstrate that the ependymins are normal constituents in the nervous system of untrained goldfish. They are specifically enriched in the brain and spinal cord, but almost entirely absent from peripheral tissues and blood serum. Methods of subcellular fractionation were used to investigate the

proteins' ultrastructural distribution in the central nervous system. Ependymins are major components in the interstitial and cerebrospinal fluids and in brain cytoplasm. The absence of ependymins from mitochondria, microsomes and purified synaptosomes indicates that they might be of glial origin and their enrichment in the extracellular fluid suggests that they might become secreted. Cells from the ependymal zone of the optic tectum were grown in culture (MAJOCHA, SCHMIDT and SHASHOUA 1982). The cultured cells secrete ependymins into the medium.

A detailed biochemical analysis revealed that ependymin β and γ are not only functionally but also structurally and metabolically related (SCHMIDT and SHASHOUA 1983): They are characterized by immunologically identical determinants (as shown by Scatchard plot analysis of antibody-antigen interaction), resemble each other in their sugar moieties, possess a very similar amino acid composition and a partly homologous amino acid sequence (demonstrated by limited proteolysis). It has been shown by radioactive tracer studies, that ependymin β is the physiological precursor molecule of ependymin γ.

We suppose that ependymins are secreted from their cells of origin into the cerebrospinal fluid and are chemically modified before they can exert their physiologica role at functional sites away from the locus of their synthesis.

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